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TECHNICAL NOTE

Fractional Urinary Clearance of Immunoreactive Lipase in Chronic Pancreatic Disease

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Summary: Using an immunoenzymatic method, we studied lipase in the serum and urine of 23 controls, 22 chronic pancreatitis patients in symptomatic remission, and in 9 patients with proven pancreatic cancer. Serum and urine lipase and its fractional urinary clearance were compared with those of amylase and immunoreactive trypsin. Lipase immunoreactivity was detectable in the urine of 81.5% of the studied subjects (controls: 82%, chronic pancreatitis: 86%, pancreatic cancer: 66%); its output was higher than the upper limit of controls in 31.8% of chronic pancreatitis and in only 1 of pancreatic cancer, and it was significantly correlated with the urinary output of trypsin ($r = 0.487$, $P < 0.001$), but not with that of amylase. A significant correlation was found between urinary output and serum levels for lipase, but not for trypsin or amylase. Fractional clearance of lipase was of the same magnitude as that of trypsin but only 0.1% that of amylase. 19% of chronic pancreatitis and pancreatic cancer showed a fractional clearance of lipase above the upper limit of controls, compared with 45% for trypsin and 3.2% for amylase. No difference in urinary clearance of the three enzymes was found between chronic pancreatitis and pancreatic cancer. In conclusion, although of no diagnostic relevance in pain-free patients with chronic pancreatic disease, this measurement can provide information on the mechanisms of renal excretion of pancreatic enzymes.

Introduction

The diagnostic value of the urinary clearance of pancreatic enzymes[1]) in acute and chronic pancreatic diseases is still a matter of wide debate. Some authors have considered an increase in the urinary clearance of amylase as an index of acute pancreatitis (1, 2) and others have considered the urinary clearance of trypsin a useful tool in the differential diagnosis between chronic pancreatitis and pancreatic cancer (3), whereas the clinical relevance of these assays has been denied by some authors both for amylase and for trypsin (4–13).

In the past, urine was investigated for lipase activity, using relatively insensitive methods. It was not detected (14, 15) because physico-chemical factors influence the urinary activity (as also found for trypsin (16–17)), or possibly due to the renal handling of the enzyme. Since a new immunoenzymatic method for lipase measurement is now available (18), the aim of this study was to investigate the levels of urinary lipase immunoreactivity, and the urinary clearance of lipase, and to compare them with amylase and trypsin serum and urine levels.

Patients and Methods

This investigation was carried out on 54 subjects (46 males and 8 females, aged 20–65 years), comprising 23 controls, 22 patients with uncomplicated chronic pancreatitis (all alcoholic in origin, pain-free since at least four weeks), and 9 with surgically and histologically proven pancreatic cancer. Controls were chosen from hospital staff and inpatients not suffering from any digestive or renal disease. The following exclusion criteria were followed: presence of urinary infection (colony forming units $> 50 \times 10^6$/l); renal impairment (blood urea nitrogen > 23 mg/dl; creatinine clearance < 40 ml/min in females, < 60 ml/min in males; proteinuria > 0.5 g/l); treatment with cytostatic drugs and/or aminoglycoside antibiotics. Urine was collected over a 12 hour period from 8 pm to 8 am in a bag containing 4 ml of a 250 g/l Na azide solution. All blood samples were taken after an overnight fast, at the end of the urine collection period, and the serum was stored at −20 °C until assay. Serum amylase was determined according to *Pierre* (19); lipase by the immunoenzymatic method proposed by *Grenner* et al. (18) (Enzygnost-Lipase, Behringwerke, Marburg, West Germany), modified by ourselves (20) (detection limit 0.5 µg/l); trypsin by the method of *Malvano* et al. (21) (Trypsik Riakit, Sorin, Saluggia, Italy). For the urine assay of lipase and trypsin, 60 ml of fresh urine were concentrated 30-fold by ultrafiltration (Amicon concentrator, Diaflo YM5 membrane; 95% cut off limit at $M_r = 5000$). Since the dilution curve of concentrated urine samples was not parallel to the standard curve of trypsin in serum, a standard curve was constructed, using the virtually trypsin-free urine of a patient with chronic pancreatitis. A detection limit as low as 0.1 µg/l with a recovery of 103 + 3.6% (x + 1 SD) was obtained with a four-fold dilution of the reagents and a 48 hour incubation period at 4 °C.

[1]) Amylase: EC 3.2.1.1
Pancreatic lipase: EC 3.1.1.3
Cathodal trypsin: EC 3.4.21.4

For urine lipase the serum method was used without modification. Serum and urine creatinine was measured by means of an automated reaction-rate method (22). The fractional clearance of the three enzymes (i. e., the ratio between their urinary clearance and that of creatinine) was evaluated according to *Levitt* et al. (1). The linear correlation index "r" was calculated after logarithmic transformation of the data.

Results

A urine lipase recovery ranging from 84 to 94% for increasing enzyme concentrations was found. When the same urine of 5 patients was concentrated on two different days a coefficient of variation ranging from 1.28 to 10.86% was found. Virtually no difference in the lipase and trypsin values was observed when the concentration was decreased stepwise from 30 to 5 fold, or after adding, to two concentrated urine samples, bovine serum albumin up to 200 g/l (to rule out concentration artifacts in patients with proteinuria).

Figure 1 shows the distribution of the individual values of the enzymes in serum. In chronic pancreatitis patients 22.7%, 13.6% and 45.4% of serum amylase, trypsin and lipase values respectively were above the upper limit found in our 23 controls, and 0%, 27.2% and 18.1% respectively were below the lower limit of controls. Figure 2 shows the distribution of enzyme levels in urine. A similar percentage of chronic pancreatitis patients (31.2 to 45.8%) showed a high urine output of each enzyme. A significant linear correlation was found between urine output and serum level for lipase ($r = 0.504$; $p < 0.001$) but not for trypsin or amylase. A significant, even if weak, correlation ($r = 0.487$; $p < 0.001$) was found only between the urinary output of trypsin and of lipase. Figure 3 shows the fractional clearance of amylase, of trypsin and of lipase. Lipase fractional clearance was very low, of the same magnitude as trypsin and roughly a thousand times less than amylase. Only 19% of chronic pancreatitis and of pancreatic cancer patients showed a lipase fractional clearance above the upper limit of our controls, compared with 45% for trypsin and 3.2% for amylase.

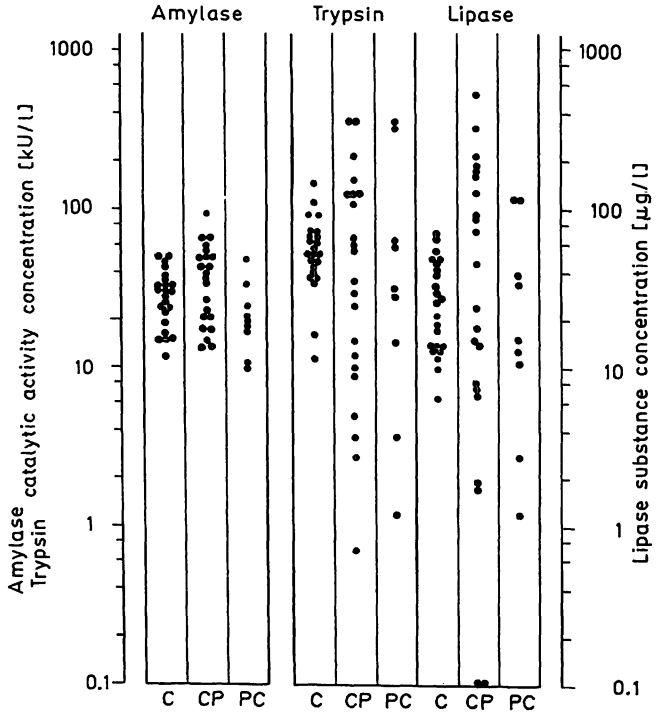

Fig. 1. Serum levels of the studied enzymes (on log scale) in controls (C) and in patients with chronic pancreatitis (CP) and with pancreatic cancer (PC).

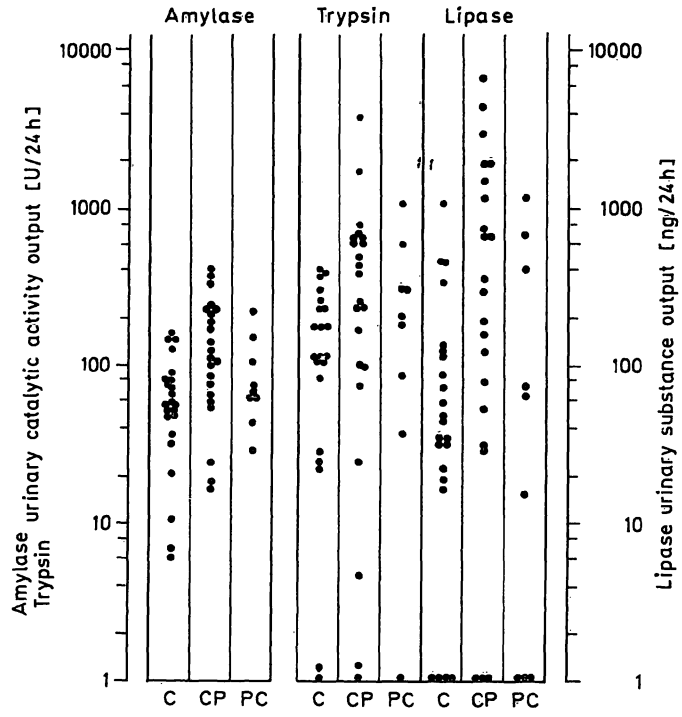

Fig. 2. Urinary outputs of the studied enzymes (on log scale). Legends as in figure 1.

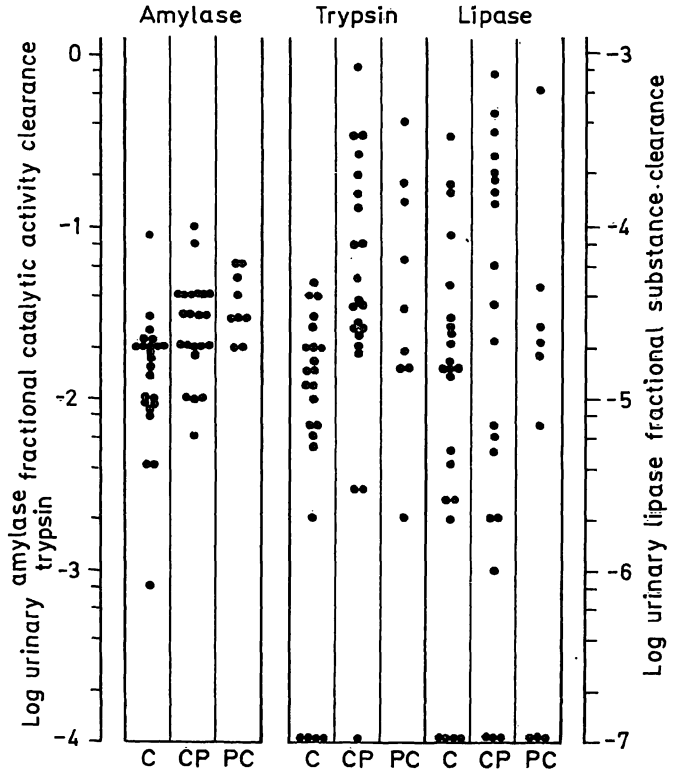

Fig. 3. Values of urinary fractional clearance of the studied enzymes (on log scale). Legends as in figure 1.

Discussion

By employing a new immunoenzymatic method for lipase (18) in 30-fold concentrated urines, we were able to measure a significant urinary output in 81.5% of the studied subjects. Very low urinary levels of this enzyme (especially if compared with amylase) were however found in the majority of our patients.

It is generally assumed that this small protein is filtered by renal glomeruli. Therefore a substantial tubular reabsorption is to be expected (23), as shown for amylase in the proximal tubule (10, 24, 25), and as concluded for lipase in man (26) on the basis of an increased urinary lipase output after selective tubular block by lysine intravenous infusion. The tubular reabsorption of lipase seems to be far more efficient than that of amylase and of the same order of magnitude as that of trypsin. On the other hand, lipase and trypsin seem to show different excretory behaviour, since a linear correlation between serum level and urinary output is found for the former and not for the latter enzyme. Abnormal values of trypsin fractional clearance are found in 45% of patients with chronic pancreatic diseases, possibly due to some form of subclinical tubular derangement (27) fairly specific for trypsin, since for lipase it occurs only in 19 percent of patients.

In conclusion, even if no clinical value can be attributed to the lipase urinary excretion in pain-free patients with chronic pancreatic diseases, this measurement can provide information of pathophysiological relevance to renal derangement in these diseases.

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